Chapter 6

Structural Characterization of Humic Substances Using Thermochemolysis with Tetramethylammonium Hydroxide

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The focus of this paper centers on a review of the recent development of the TMAH thermochemolysis as a rapid, low-cost, and easily implemented technique for the structural analysis of humic substances. Pyrolysis, or rather chemolysis, in the presence of TMAH has been used for the structural characterization of humic substances from different origins. The procedure methylates carboxylic groups and hydroxyl groups, rendering the chemolytic products more amenable to chromatographic separation. The examination of humic substances with this technique reveals the presence of a series of benzenecarboxylic acid methyl esters as well as long-chain fatty acid methyl esters and dimethyl esters. The methylated structures produced by TMAH differ dramatically from those obtained by conventional pyrolysis, calling into question the recently proposed structure of humic acids which are based mostly on conventional pyrolysis. The procedure consists mainly of a thermally assisted chemolysis rather than true pyrolysis, and consequently is effective at subpyrolysis temperatures. This means that the procedure can also be easily implemented in sealed glass tubes. In general, the studies to date have demonstrated that this technique provides excellent preservation of the original structures containing carboxyl and hydroxyl groups in lignin monomers owing to protection of the functional groups from thermal reactions. The TMAH thermochemolysis also induces β-ether bond lysis in lignin. This is significant because the procedure shows potential in characterizing lignin-derived compounds in much the same way as the CuO oxidation procedure.

Characterization of complex organic matter like humic substances (HS) is a formidable task (*1*). A variety of destructive and non-destructive methods have been applied. Among the non-destructive methods, spectroscopic methods, such as NMR and FT-IR have proven to be very useful in providing information about the structure of these materials as a whole. Of these, NMR has proven to be the best method for bulk characterization, especially solid-state ^{13}C NMR, where the relative contribution of specific carbon types can be made (*2*). Different structural parameters (aromaticity,

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carboxyl content, etc.) could be determined directly. However, for more detailed information on the structure of HS at a molecular level, degradative methods need to be applied. Among them, wet chemical methods and pyrolytic methods have been widely used in the past years to get insight into the chemical constitution of these materials (*1,3,4*).

Wet chemical methods have the advantage of providing information on the molecular fragments comprising HS, but exhibit several disadvantages as they often require high quantities of sample, they interfere with the sample, and they sometimes are very drastic. Wet chemical degradations such as the CuO method of Hedges and Ertel (*5*) can identify components derived from lignin and other biopolymers (*3,6*).

Pyrolytic techniques coupled with mass spectrometry (*7*) and gas chromatography-mass spectrometry (*4,8*) have proved to be particularly useful in structural characterization of HS due to detailed information obtained at a molecular level and the ease of sample preparation. They usually require low amounts of sample and no chemical pretreatments are needed, avoiding possible contamination from laboratory manipulations. In many instances pyrolysis is very reproducible and the results can be interpreted both qualitatively and quantitatively, and finally, it is easily performed in a pyroprobe. The thermal degradative products can be then related to original moieties present in the structure of the HS. The pyrolysis of polar macromolecular materials is well known to produce volatile polar products which can be chromatographed; however, very polar products also produced are often so polar as to remain attached to the column and of insufficient volatility to be chromatographed. These products are simply not observed and remain unquantified. This is because the high polarity of products leads to further decomposition before volatilization, prevents transfer from the pyrolysis unit to the GC column, or makes it impossible to pass through a GC column. Also, some significant structural moieties can be heavily modified by unwanted thermal reactions which may lead to misinterpretation of the structure of the HS (*9-12*).

Recently, a new technique has been introduced for the characterization of polar macromolecules such as HS. This technique was originally introduced as pyrolysis with *in situ* methylation (*13*). The presence of a gaseous methylating agent, tetramethylammonium hydroxide (TMAH), at the point of pyrolysis greatly assists in converting polar products to less polar derivatives which are more amenable to chromatographic separation (*9,13-17*). This procedure avoids decarboxylation and produces the methyl esters of carboxylic acids and methyl ethers of hydroxyl groups, rendering many of the polar products volatile enough for gas chromatographic analysis. Thus, it is possible to separate and detect many more structurally significant products than that observed previously by conventional pyrolysis-GC-MS. This technique for the analysis of HS has greatly enhanced product yields and produces some products not observed by conventional pyrolysis (*9,11,15-17*).

Challinor (*13,18,19*) first introduced this technique for the simultaneous pyrolysis and methylation of phenolic polymers, and since then, it has been applied to different biopolymers (*14,20,21*), humic materials (*9-11,15-17,22-25*), asphaltenes and kerogens (*26,27*) and natural and fosil resins and resinites (*28,29*).

Several authors (*9,12,14,16,30*) have pointed out that the reaction involved in the TMAH/pyrolysis scheme is one of chemolysis rather than pyrolysis. Because the procedure is more likely a thermally-assisted chemolysis or "thermochemolysis", sub-pyrolysis temperatures of 300°C have also been found to effectivelly produce a suite of products similar to that observed at higher pyrolysis temperatures (*16,21*). Moreover, in a recent paper by McKinney *et al.* (*31*), a procedure was outlined for the characterization of lignin at subpyrolysis temperatures of 300°C in the presence of TMAH using sealed pyrex tubes. Since then, this technique has been extended to the characterization of lignin in fresh and degraded woods (*32*) and in coalified woods (*33*).

Application of the TMAH/Thermochemolysis Procedure to Humic Materials

As noted above, the use of TMAH/thermochemolysis for the structural characterization of different humic materials have been applied in two different forms: pyrolysis of the humic materials in the presence of TMAH or a confined reaction of the humic materials with the TMAH in a sealed glass ampoule at lower temperatures. Both methods release the same types of compunds.

Lignin is an important component in the structure of most humic materials and many lignin-derived compounds are released in the TMAH/thermochemolysis procedure. A model for a gymnosperm lignin is depicted.in Figure 1 to help the understanding of much of the discussion refered to the lignin structure along the text. This structure is essentially a modified model of Adler (*34*) for spruce wood, and it is important to note several structural features for further reference. While the gymnosperm lignin is made up exclusively of guaiacyl units, angiosperm and grass lignins contain also syringyl and *p*-hydroxycoumaryl units. Most lignin units are linked to each other via β-O-4 linkages but other linkages included β-β, α-O-4, β-5 and 5-5' linkages using the standard notation for lignin units (Figure 1).

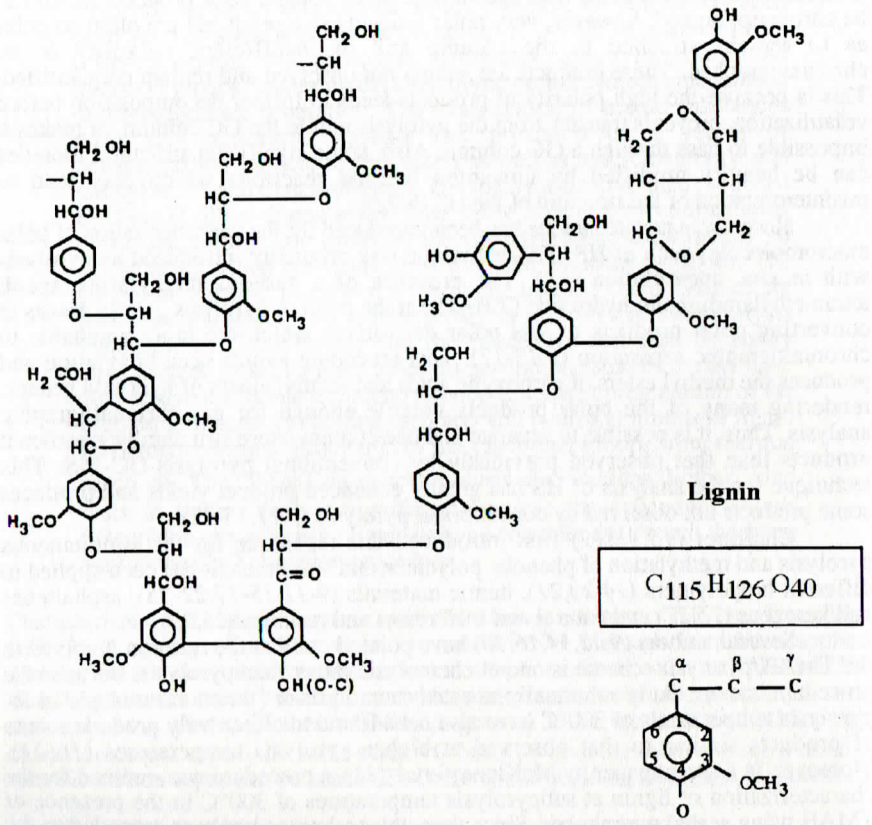

Figure 1.- Structural model for a modern lignin from gymnosperm (modified from Adler, *34*). The numbering convention is shown for a typical structural unit of lignin. Reproduced with permission from reference 34. Copyright 1990 Elsevier Science

Pyrolysis in the Presence of TMAH. A wide set of Fulvic Acids (FA), Humic Acids (HA) and related materials from different origins have been thus far studied using the TMAH/thermochemolysis procedure (*9,15-17,22,24,25*). The TMAH procedure has mainly been performed in pyroprobe units, in much the same way as the conventional flash pyrolysis using either quartz tubes and/or a platinum coil, by mixing the humic material with a few drops of the reagent prior to heating.

Characterization of Fulvic Acids. Figure 2 shows the chromatogram of the products released after the pyrolysis of the FA isolated from a water-logged peatland, both in the absence and in the presence of TMAH. Peaks identifications are in Table I. The flash heating of the FA in the presence of TMAH yields mainly derivatives from polysaccharides and lignin which have become incorporated into the FA macromolecular structure. Methyl esters of aliphatic carboxylic acids were also released. This procedure yields products that are not released with conventional pyrolysis, because they are too polar or too reactive. The most striking feature is the identification of aromatic acids, as methyl esters, which have been previously reported as being building blocks of the FA structure in models based upon oxidative degradations (*1*). Saiz-Jimenez *et al.* (*15,22*) and Martin *et al.* (*9*) published the only studies thus far in which pyrolysis in the presence of TMAH is used for the structural characterization of FAs. They found that large quantities of aromatic acids were released from different soil and lake FAs, suggesting that these compounds might represent final steps in the oxidation of the side-chain during microbial degradation of lignins. Benzenecarboxylic acid moieties have not previously been released from humic substances upon conventional pyrolysis techniques due to decarboxylation

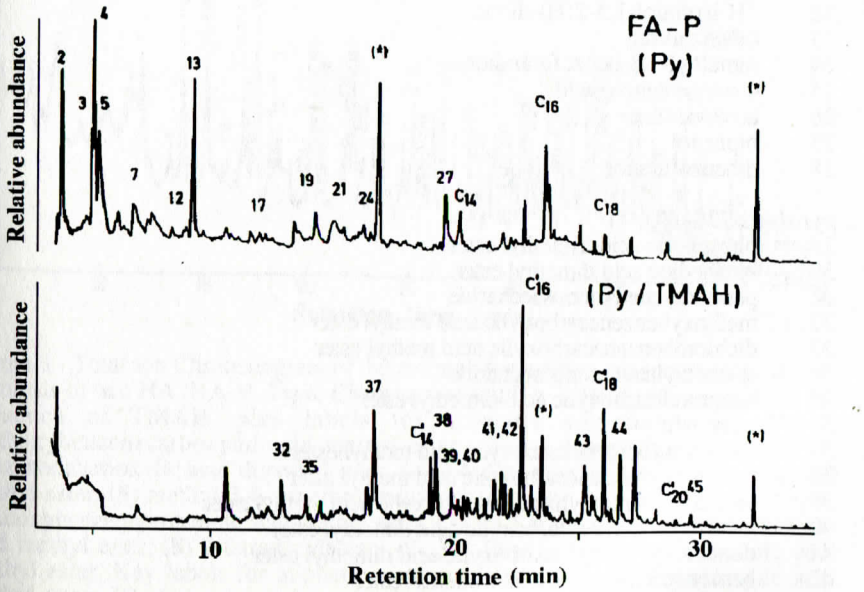

Figure 2.- Total Ion Chromatogram of the thermal degradative products obtained after pyrolysis of the FA isolated from a water-logged peatland, in the absence (Py) and in the presence of TMAH (Py/TMAH). For peak identifications refer to Table I. Contaminants are noted by (*) and the peaks labeled Cn are fatty acids and C'n are fatty acid methyl esters. Reproduced with permission from reference 9. Copyright

Table I. Main thermal degradative products identified in the pyrolysates of the fulvic acids isolated from a water-logged peatland Reproduced with permission from reference 9. Copyright 1994 Elsevier Science—Netherlands.

Conventional pyrolysis	
1	acetic acid
2	furfural
3	methylfurfural
4	dimethyltrisulphide
5	phenol
6	2-furancarboxylic acid
7	cresol
8	guaiacol
9	furanmethanol
10	benzenediol
11	2-methyl-1H-isoindol-1,3(2H)-dione
12	2-methyl-3-hydroxypyran-4-one
13	dimethyltetrasulphide
14	benzoic acid
15	2-butenoic acid
16	benzenediol
17	1,3-benzofurandione
18	2,2'-bifuran
19	methyl-1,3-benzofurandione
20	benzopyran-2-one
21	methoxy-1,3-benzofurandione
22	1H-isoindol-1,3-2(H)-dione
23	dibenzofuran
24	dimethyl-1,3-benzofurandione
25	4-oxopentenoic acid
26	acetovanillone
27	biphenol
28	dibenzofuranol
pyrolysis/TMAH	
29	phosphoric acid trimethyl ester
30	butanedioc acid dimethyl ester
31	permethylated monosaccharide
32	methoxybenzenecarboxylic acid methyl ester
33	dichlorobenzenecarboxylic acid methyl ester
34	permethylated monosaccharide
35	benzenedicarboxylic acid dimethyl ester
36	unknown
37	dimethoxybenzenecarboxylic acid methyl ester
38	trimethoxybenzenecarboxylic acid methyl ester
39	dimethoxydimethylbenzenecarboxylic acid methyl ester
40	methoxybenzenedicarboxylic acid dimethyl ester
41	dimethoxybenzenedicarboxylic acid dimethyl ester
42	benzenetricarboxylic acid trimethyl ester
43	methoxybenzenetricarboxylic acid trimethyl ester
44	benzenetetracarboxylic acid tetramethyl ester
45	methoxybenzenetetracarboxylic acid tetramethyl ester
46	benzenepentacarboxylic acid pentamethyl ester

process. The use of pyrolysis in the presence of TMAH avoids decarboxylation by protecting the carboxyl group, and releases them as their methyl esters. The aboveauthors agreed in that the benzenecarboxylic acids would represent structural constituents of FAs. In addition, these results are also consistent with the high carboxyl carbon contents reported in the structure of FAs as measured by non-degradative ^{13}C-NMR (*35*) and other methods (*1*).

Characterization of Humic Acids. Similar compounds are also released from soil HA and Figure 3 shows the chromatograms of the compounds released after pyrolysis in the presence of TMAH for two selected HA (*17*). High proportions of long-chain fatty acid methyl esters, as well as phenolic derivatives and aromatic acid methyl esters, were released. The released compounds might represent structural components of the humic macromolecule, as also suggested by different authors

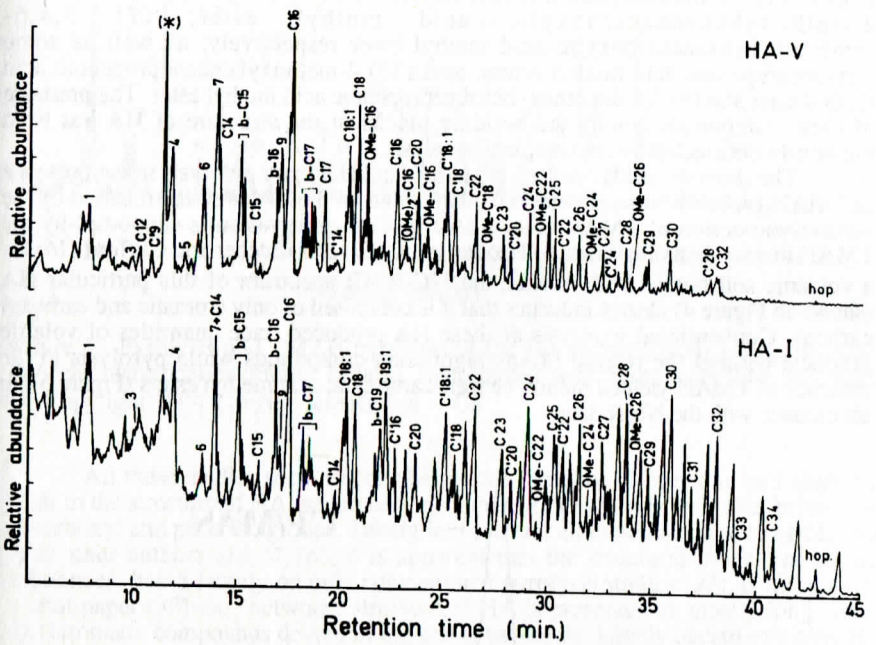

Figure 3.- Total Ion Chromatogram of the thermal degradative products obtained after pyrolysis of two HA (HA-V: *Typic Chemoxerert* and HA-I: *Histic Humaquept*) in the presence of TMAH. Key labels for aromatic compounds are:.(**1**) 4-methoxybenzenecarboxylic acid methyl ester, (**2**) 1,3,4-trimethoxybenzene, (**3**) benzenedicarboxylic acid dimethyl ester, (**4**) 3,4-dimethoxybenzenecarboxylic acid methyl ester, (**5**) methyl-3,4-dimethoxybenzenecarboxylic acid methyl ester, (**6**) 4-methoxybenzenepropenoic acid methyl ester, (**7**) 3,4,5-trimethoxybenzenecarboxylic acid methyl ester, (**8**) 2-tetradecylfuran, (**9**) 3,4-dimethoxybenzenepropenoic acid methyl ester. Key labels for aliphatic compounds are: (Cn) monocarboxylic acid methyl esters, (Cn:1) unsaturated monocarboxylic acid methyl esters, (b-Cn) branched monocarboxylic acid methyl esters, (C'n) dicarboxylic acid dimethyl esters, (C'n:1) unsaturated dicarboxylic acid dimethyl esters, (OMe-Cn) methoxymonocarboxylic acid methyl esters, (OMe$_2$-Cn) dimethoxymonocarboxylic acid methyl esters, (OMe-C'n) methoxydicarboxylic acid dimethyl esters, (hop) hopanoids. Reproduced with permission from reference 17. Copyright 1995 Elsevier Science–Netherlands.

(*10,12,16,17*).The different series of aliphatic acids released after pyrolysis in the presence of TMAH consisted mainly of C_6-C_{34} mono- and dicarboxylic acids as well as their methoxylated counterparts. The different series of aliphatic acids may be chemically bound to the HA matrix in a form similar to that suggested by Schnitzer and Neyroud (*36*). The α,ω-alkanoic diacids may act as bridges in the macromolecule, their content being related to the cross-linking state of the macromolecular network. Homologous series of fatty acid methyl esters and a,w-alkanedioic acid methyl esters, and triterpenoid compounds with ursane, oleanane and hopane skeletons were also detected in the structure of different HAs upon pyrolysis in the presence of TMAH by different authors (*10,16,17,25*).

Different phenolic derivatives with lignin-related structures were also released. Among them, we note the presence of some benzenecarboxylic acids, some of them being lignin structures with *p*-hydroxycoumaryl; guaiacyl and syringyl skeletons, peaks (1) 4-methoxybenzenecarboxylic acid methyl ester, (4) 3,4-dimethoxybenzenecarboxylic acid methyl ester, (7) 3,4,5-trimethoxybenzenecarboxylic acid methyl ester respectively, as well as some benzenepropenoic acid methyl esters, peaks (6) 4-methoxybenzenepropenoic acid methyl ester and (9) 3,4-dimethoxybenzenepropenoic acid methyl ester. The presence of these compounds among the building blocks of the structure of HA has been previously undetected by conventional pyrolysis.

The aromatic acids released from different HA upon pyrolysis in the presence of TMAH probably represent original components of the HA structure released by the thermolytic action of TMAH (*10,12,16,17*). This observation is supported by the TMAH thermochemolysis data of Hatcher *et al.* (*23*) and Hatcher and Clifford (*16*) for a volcanic soil humic acid. In fact, the ^{13}C-NMR spectrum of this particular HA (shown in Figure 4) clearly indicates that it is composed of only aromatic and carboxyl carbons. Conventional pyrolysis of these HA produced trace quantities of volatile products without the release of any significant compounds while pyrolysis in the presence of TMAH yielded mainly benzenecarboxylic acid methyl esters (Figure 5), in accordance with the NMR data.

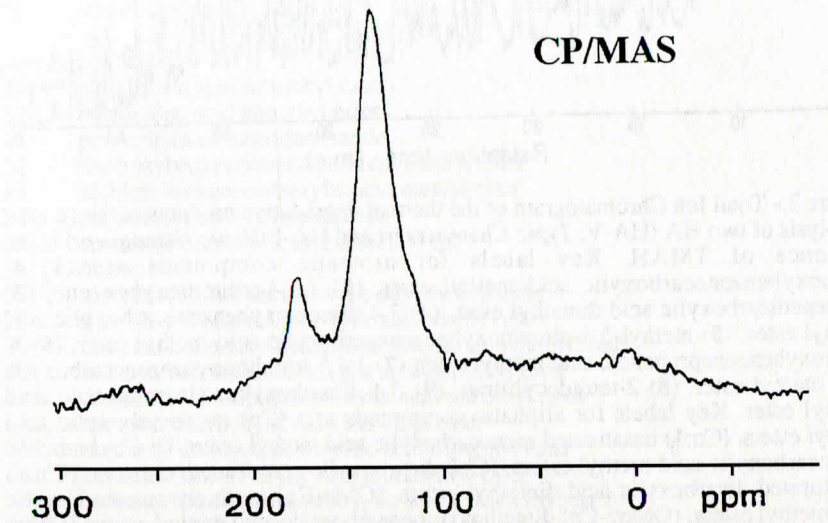

Figure 4.- Solid-state ^{13}C-NMR spectrum of the HA isolated from a volcanic soil Reproduced with permission from reference 16. Copyright 1994 Elsevier Science

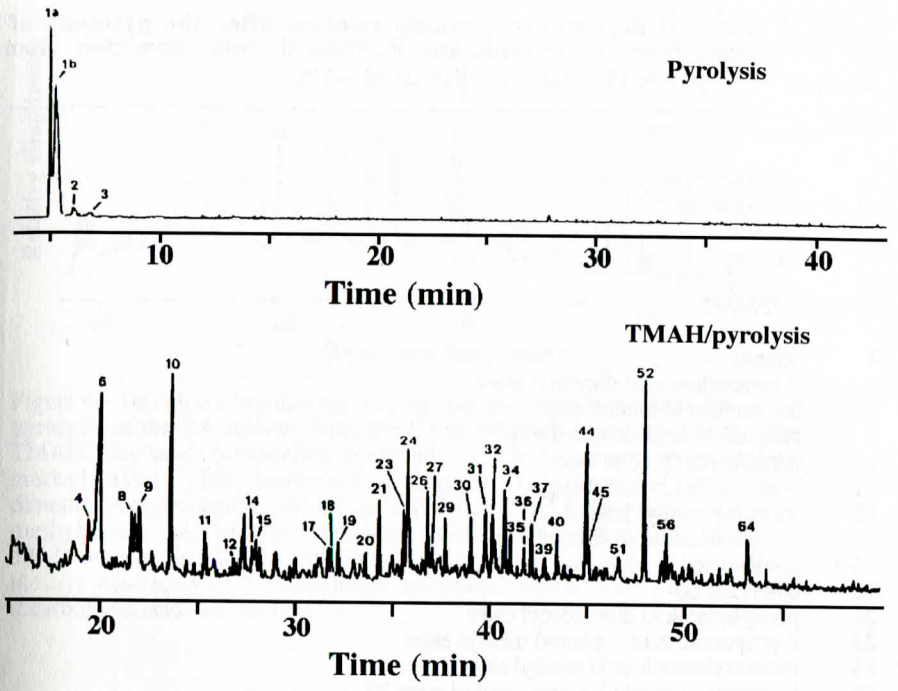

Figure 5.- Pyrolysis and TMAH/pyrolysis of the HA isolated from a volcanic soil. For peak identification refer to Table II. Reproduced with permission from reference 16. Copyright 1994 Elsevier Science Ltd.—UK.

All these studies seem to corroborate the presence of aromatic and aliphatic acids in the structure of HA, which become apparent in pyrolysis only after protection of carboxyl and phenolic groups. Taking into account all these data, and in agreement with other authors (*10,12,16*), it is apparent that the structural model for humic substances, based largely on only conventional pyrolysis studies (*37*), is inaccurate. In that paper (*37*) the "network" structure of HA is presented as mostly long-chain alkylaromatic compounds devoid of oxygen constituents, largely because no oxygen-containing compounds were reported among the pyrolysis products dominated by alkyl aromatic compounds. The elemental analysis, largely ignored, indicated the presence of 33-34% of oxygen in the HA investigated. The same authors have now revised their original structure, with the inclusion of oxygen in the form of carboxyls, phenolic and alcoholic hydroxyls, carboxyl esters and ethers (*38,39*) attached to their original "network". The model is now more consistent with the elemental data but is clearly inconsistent with the results derived from the pyrolysis data originally presented by these authors.

Pyrolysis in the presence of TMAH has also been applied to the structural characterization of HAs isolated from low-rank coals. Figure 6 shows the chromatogram of the compounds released after the TMAH/pyrolysis of the HA isolated from a humic coal from Konin (Poland). A large variety of components were released, the lignin-derived phenol derivatives and aliphatic acid methyl esters being the most prominent. A series of fatty acid methyl esters were identified in the range from C_{10} to C_{34}, with maxima at C_{16} and C_{18} showing an even-over-odd

Table II. Thermal degradative products released after the pyrolysis of the HA isolated from the volcanic ash. Reproduced with permission from reference 16. Copyright 1994 Elsevier Science Ltd.—UK.

Conventional pyrolysis	
1a	chlorotrifluoroethane
1b	chloroethane
2	benzene
3	toluene
Pyrolysis/TMAH	
4	phenol
6	2-butanedioc acid dimethyl ester
8	hexanoic acid methyl ester
9	butanedioc acid dimethyl ester
10	benzoic acid methyl ester
11	nonanoic acid methyl ester
12	methoxy methyl phenol
14	1-methyl-2,5-pyrrolidinedione
15-19	unidentified
20	C6H12)2 (t)
21	phosphoric acid dioctadecyl ester
23	2-propenoic acid-3-phenyl methyl ester
24	methoxybenzoic acid methyl ester
26	L-proline, 1-methyl-5-oxo, methyl ester (t)
27	dimethylundecanoic acid methyl ester (t)
29	1,3,5-triazine-2,4,6 (1H,3H,5H) trione,1,3,5-trimethyl (t)
30	ninhydrin
31	benzenedicarboxylic acid dimethyl ester
32	benzenedicarboxylic acid dimethyl ester
34	benzenedicarboxylic acid dimethyl ester
35	2,4 (1H,3H)-pyrimidinedione,1,3,5-trimethyl ester
36	methyl trimethoxy phenol (t)
37	tetradecanoic acid methyl ester
39	dimethoxybenzaldehyde
40	3,5-dimethoxybenzoic acid methyl ester
44	dimethoxybenzoic acid dimethyl ester
45	methyltetradecanoic acid methyl ester
51	unidentified
52	hexadecanoic acid methyl ester
56	trimethoxybenzoic acid methyl ester
64	unidentified

(t) tentatively identified compounds.

predominance. This series may be derived from ester bound long-chain carboxylic acids linked to the HA macromolecular network via ester bonds. A series of *n*-alkanes/*n*-alkenes, with chain lengths up to C_{33} and no even-over-odd predominance, was also released, indicating the presence of significant amounts of polyalkyl components in the structure of this HA. The fact that the series of *n*-alkanes/*n*-alkenes

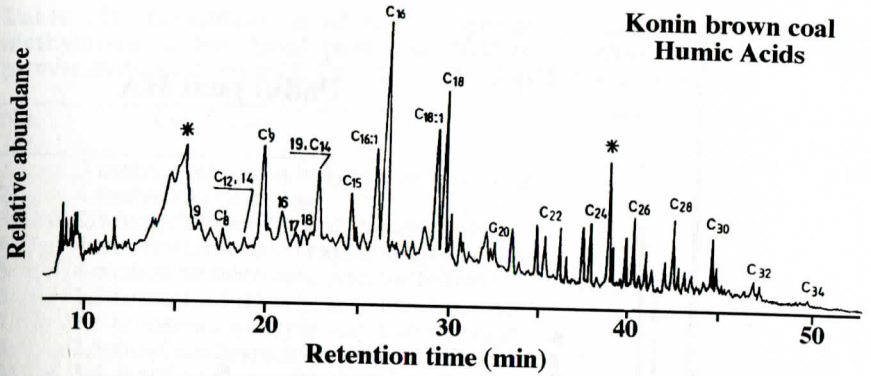

Figure 6.- Total Ion Chromatogram of the thermal degradative products obtained after pyrolysis of the HA isolated from the Konin (Poland) brown coal in the presence of TMAH. Key labels for aromatic compunds are: (9) 4-methoxybenzenecarboxylic acid methyl ester, (14) benzenedicarboxylic acid dimethyl ester, (16) 3,4-dimethoxybenzenecarboxylic acid methyl ester, (17) 3,4-dimethoxybenzeneacetic acid methyl ester, (18) 4-methoxycinnamic acid methyl ester, (19) 3,4,5-trimethoxy-1-ethylbenzene. Key labels for aliphatic compounds are: (Cn) monocarboxylic acid methyl esters, (Cn:1) unsaturated monocarboxylic acid methyl esters, (C'n) dicarboxylic acid dimethyl esters.

and long-chain carboxylic acid methyl esters are released together upon pyrolysis in the presence of TMAH seems to suggest that the long-chain carboxylic acids may arise from different aliphatic moieties than the *n*-alkanes/*n*-alkenes. This is also supported by the fact that there is no parallel between the chain length distribution of *n*-carboxylic acids and the distributions of hydrocarbons in the HA.

Again, a striking feature in this study of coal-derived HA is the release of lignin-derived phenol derivatives bearing carboxyl groups in the side-chain (benzenecarboxylic acid, benzeneacetic acid and benzenepropionic acid), as their methyl esters, as well as aliphatic acid methyl esters, which have not been observed previously by conventional pyrolysis. Del Rio *et al.* (*24*) also conducted TMAH/pyrolysis of HA isolated from a peat and a lignite. Figure 7 shows the chromatograms of the compounds released after TMAH/pyrolysis of the HA isolated from Padul peat and PGR lignite. An increasing amount of benzenecarboxylic acid methyl esters and benzylic ketones were observed in the TMAH thermochemolysis products of HA from coals of increasing rank. This fact is in agreement with the model proposed by Hatcher (*40*) for the transformation of lignin up to low-rank coal levels. According to this model, the content of carboxyl groups in the lignin macromolecule increases during the first stages of the coalification process, and reaches the maximum at the lignite stage. McKinney and Hatcher (*33*), in studies of TMAH/thermochemolysis of gymnosperm coalified woods of increasing rank, also observed that as the lignin biopolymer is altered and oxidized through time, carboxylic acid methyl ester functionalities increase in relative abundance. Oxidation of the C-3 side chain of the lignin structure would produce the benzenecarboxylic acids and the benzylic ketones identified.

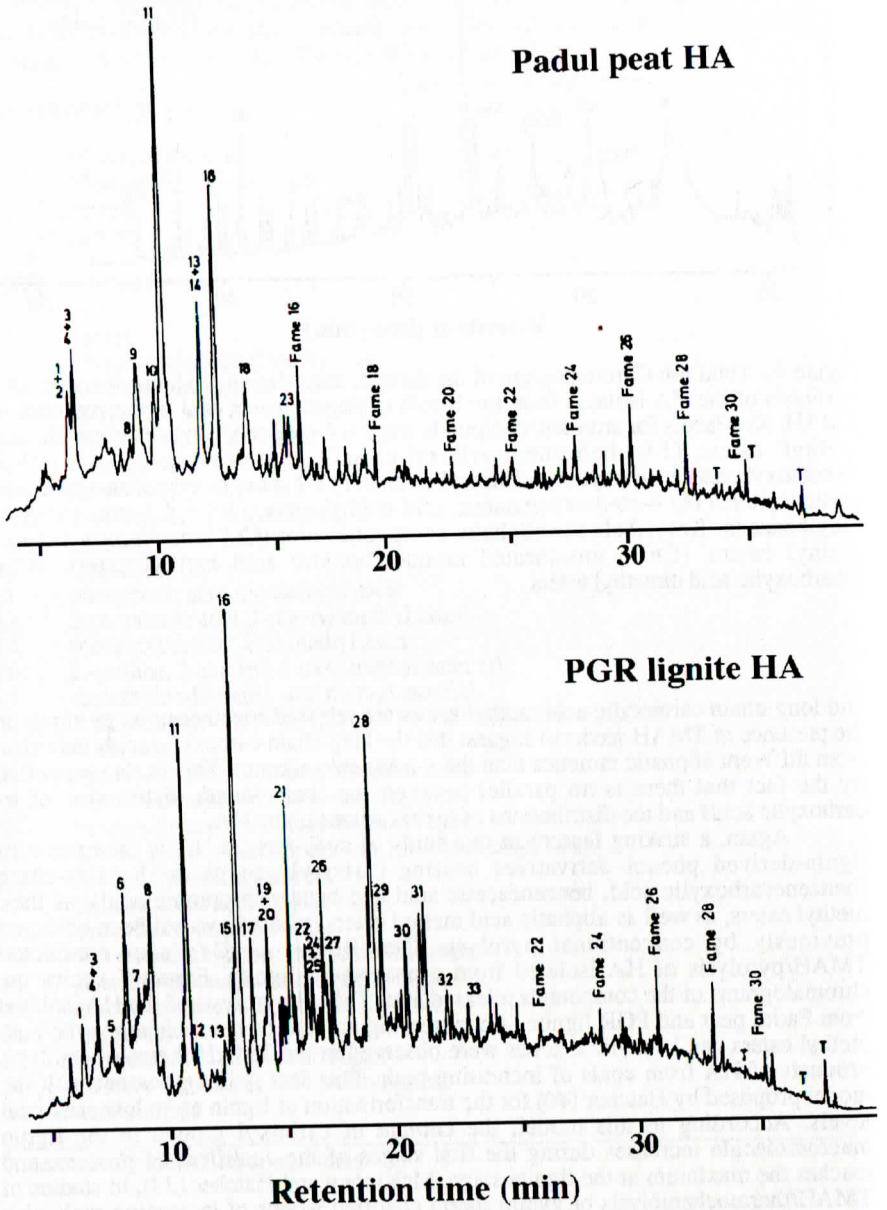

Figure 7.- Total Ion Chromatogram of the thermal degradative products obtained after pyrolysis of the HAs isolated from Padul peat and PGR lignite in the presence of TMAH. Peaks are identified in Table III. Reproduced with permission from reference 24. Copyright 1994 Elsevier Science Ltd.—UK.

Table III. Identification of the compounds released after pyrolysis-methylation of the Padul peat and PGR lignite HAs. Reproduced with permission from reference 24. Copyright 1994 Elsevier Science Ltd.—UK.

No.	Compound
1	3-methoxybenzenecarboxylic acid, methyl ester
2	4-methoxyacetophenone
3	2,5-furandicarboxylic acid, dimethyl ester
4	4-methoxybenzenecarboxylic acid, methyl ester
5	4-methoxybenzeneacetic acid, methyl ester
6	1,2-benzenedicarboxylic acid, dimethyl ester
7	1,3-benzenedicarboxylic acid, dimethyl ester
8	2,5-thiophenedicarboxylic acid, dimethyl ester
9	3,4-dimethoxybenzenemethanol, methyl ether
10	3,4-dimethoxyacetophenone
11	3,4-dimethoxybenzenecarboxylic acid, methyl ester
12	3,4-dimethoxybenzeneacetic acid, methyl ester
13	3-(4-methoxyphenyl)-2-propenoic acid, methyl ester
14	3,4,5-trimethoxyacetophenone
15	3-methoxybenzene-1,2-dicarboxylic acid, dimethyl ester (t)
16	3,4,5-trimethoxybenzenecarboxylic acid, methyl ester
17	4-methoxybenzene-1,3-dicarboxylic acid, dimethyl ester (t)
18	3,4,5-trimethoxybenzenemethanol, methyl ether
19	2,3,4-trimethoxybenzenecarboxylic acid, methyl ester
20	2-methoxybenzene-1,5-dicarboxylic acid, dimethyl ester (t)
21	1,2,4-benzenetricarboxylic acid, trimethyl ester (t)
22	3,4-dimethoxybenzene-1,2-dicarboxylic acid, dimethyl ester (t)
23	3-(3,4-dimethoxyphenyl)-2-propenoic acid, methyl ester
24	1,3,5-benzenetricarboxylic acid, trimethyl ester (t)
25	4,5-dimethoxybenzene-1,3-dicarboxylic acid, dimethyl ester (t)
26	4,5-dimethoxybenzene-1,2-dicarboxylic acid, dimethyl ester (t)
27	3-methoxybenzene-1,2,4-tricarboxylic acid, trimethyl ester (t)
28	2-methoxybenzene-1,3,5-tricarboxylic acid, trimethyl ester (t)
29	5-methoxybenzene-1,3,5-tricarboxylic acid, trimethyl ester (t)
30	1,2,3,4-benzenetetracarboxylic acid, tetramethyl ester (t)
31	1,2,4,5-benzenetetracarboxylic acid, tetramethyl ester (t)
32	5-methoxybenzene-1,2,3,4-tetracarboxylic acid, tetramethyl ester (t)
33	2-methoxybenzene-1,3,4,5-tetracarboxylic acid, tetramethyl ester (t)

(t) tentatively identified compounds.

It is likely that the benzenecarboxylic acids are derived from lignin units where the α-carbon of the side-chain has been oxidized to a carboxyl group (*32*), and different studies have interpreted the structures and distributions of the methyl esters of benzenecarboxylic acids as indicating that these structural units exist as either free or ester-bound structures in the HA (*10,16,17,24*). However, it has recently become clear that certain benzenecarboxylic acids are produced from unoxidized lignin moieties by the TMAH reagent in the course of the reaction at elevated temperatures (*41*). Examination of the products from the TMAH thermochemolysis of a model lignin dimer, free of any carboxylic functionality, showed the production of relatively large amounts of methylated benzenecarboxylic acid derivatives. These authors

concluded that the release of benzenecarboxylic acids after TMAH/thermochemolysis of HA are only partially indicative of the presence of oxidized lignin units in the HA structure, and due caution is warranted in interpreting them as being derived exclusively from constituent structural entities.

TMAH/Thermochemolysis in Sealed Glass Tubes. It has recently been established that the TMAH procedure can also be conducted at sub-pyrolysis temperatures of 300°C (*16,21*). The same distribution of products are observed at pyrolysis and sub-pyrolysis temperatures, supporting the idea that the predominant effect of TMAH is a saponification/esterification reaction, as also pointed out by several other authors (*9,12,14,30*). Further investigations have prompted a study of the feasibility of carrying out the procedure at 300°C in a glass microreactor constructed of nothing more than a glass ampoule (*31*), without the need of specialized pyrolysis equipment. Therefore, the TMAH thermochemolysis procedure becomes a rapid, low-cost, and easily implemented technique for the analysis of HS. This procedure can be easily implemented in any laboratory having gas chromatographic capabilities. This is a potentially valuable advantage, because it makes the technique readily available to most geochemical laboratories.

The procedure of TMAH thermochemolysis in sealed glass ampoules has already been applied to different materials such as lignins, fresh and degraded wood samples and coalified woods (*31-33*). This procedure still induces cleavage of the β-O-4 bonds in lignin, releasing a distribution of products similar to that obtained from the CuO oxidation procedure, except for the fact that the CuO procedure mainly produces lignin monomers which are either the acid, aldehyde or methyl ketone derivatives. In the TMAH procedure, several phenol propanoids having one, two or three methoxyls at the propanoid side-chain are also released. Therefore, similar ratios as those calculated in the CuO oxidation procedure can be calculated here. In particular, this procedure appears to be more sensitive for calculating the acid/aldehyde (Ad/Al) ratios, displaying a larger dynamic range than that observed by CuO oxidation (*32*).

The TMAH thermochemolysis in sealed glass ampoules has been found to be an excellent approach for the characterization of other humic-like materials, such as dissolved organic matter (DOM). Several DOM samples, ranging from riverine systems to coastal oceans and oceanic samples have been recently studied using TMAH thermochemolysis procedure. Figure 8 shows the products released after the TMAH thermochemolysis of the Suwannee river DOM. Different lignin-derived phenol derivatives were identified among the released products, and these originate from the cleavage of the β-O-4 bonds from incorporated lignin-derived structures. The major products identified are the benzoic acid derivatives of the guaiacyl and syringyl structures, 3,4-dimethoxybenzenecarboxylic acid methyl ester (peak **37**) and 3,4,5-trimethoxybenzenecarboxylic acid methyl ester (peak **44**) respectively, with minor amounts of the cinnamyl derivative 4-methoxybenzenecarboxylic acid methyl ester (peak **17**). This means that this DOM is mainly composed of oxidized lignin moieties at the α-carbon of the side-chain. Benzeneacetic and benzenepropionic acid methyl esters were also found in the Suwannee river DOM. Benzoic acid derivatives were also identified as major compounds in the DOM from decomposing *Juncus effusus* upon pyrolysis in the presence of TMAH (*42*). A striking feature observed in the Suwannee river DOM is the identification, as major peaks, of 1,3,5-trimethoxybenzene (peak **21**) and 2,4,6-trimethoxytoluene (peak **25**), which are structures not related to lignin. Similar compounds, however, have been previously found as products from the TMAH thermochemolysis of cutan (*43*), the highly aliphatic and resistent biopolymer present in leaf cuticles from certain plants (*44*), which might indicate the contribution of this biopolymer to the structure of the Suwannee river DOM.

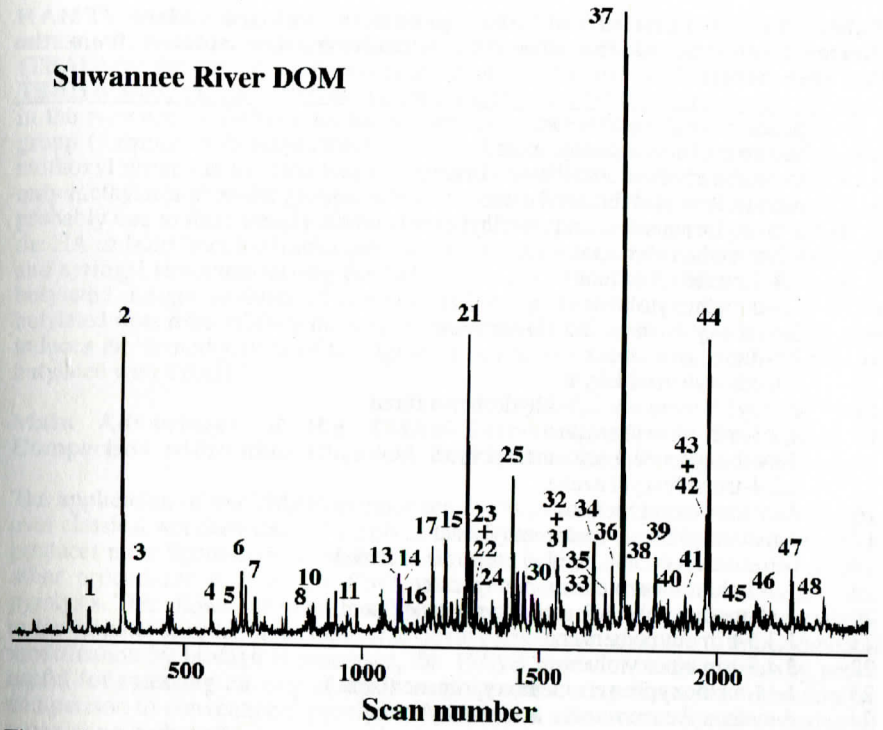

Figure 8.- Total Ion Chromatogram of the thermal degradative products obtained after TMAH/thermochemolysis in sealed glass tubes, at 250°C, of the dissolved organic matter (DOM) isolated from the Suwannee River. Peaks are identified in Table IV.

The Use of Tetrabutylammonium Hydroxide as an Alternative Alkylating Reagent

While the TMAH method is shown here to yield mono-, di-, and trimethoxyphenols derived from the three primary lignin units, it can also produce the same phenols from another component of vascular plant material, namely tannins. Tannins contain di- and trihydric phenols which can be expected to be methylated with TMAH. This is especially true with the hydrolyzable tannins which contain gallic acid esterified to sugars. The TMAH will clearly produce trimethoxy benzoic acid methyl ester, a major product also expected from TMAH/thermolysis of lignin from angiosperms. The non-hydrolyzable tannins contain dihydric phenols but these are carbon-bonded to the proanthocyanidin units and are unlikely to form products which can be similar to those from lignin. Similarly, a very well-documented consequence of the coalification process, such as the demethylation of methoxyl groups of guaiacyl and syringyl structures of lignin and the formation of the corresponding catechols (*45,46*) can also be highly biased by the use of TMAH. This reagent will produce the methyl ethers of the free phenolic groups of both methoxyphenols and dihydric phenols; the latter cannot be differentiated from the original methoxy phenolic structures in lignin.

Table IV. Identities of the products relased after TMAH thermochemolysis of the dissolved organic matter isolated from the Suwanee river.

1	pentanoic acid methyl ester
2	butanedioic acid dimethyl ester
3	methylbutanedioic acid dimethyl ester
4	pentanedioic acid dimethyl ester
5	2-methylpentanediic acid dimethyl ester
6	1,2-dimethoxybenzene
7	1,4-dimethoxybenzene
8	3,4-dimethoxytoluene
9	3-methoxy-4-acetyl-2-5(H)-furanone
10	2,5-dimethoxytoluene
11	4-methoxybenzaldehyde
12	2-vinyl-5-methoxy-2,3-dihydrobenzofuran
13	1,2,3-trimethoxybenzene
14	3-methoxybenzoic acid, methyl ester
15	1,2,4-trimethoxybenzene
16	4-methoxyacetophenone
17	4-methoxybenzoic acid, methyl ester
18	benzeneacrylic acid, methyl ester (*cis* or *trans*)
19	3,4-dichlorobenzoic acid, methyl ester
20	1-(4-methoxyphenyl)-methoxypropane (isom.)
21	1,3,5-trimethoxybenzene
22	3,4,5-trimethoxytoluene
23	1-(4-methoxyphenyl)-methoxypropane (isom.)
24	4-methoxybenzeneacetic acid, methyl ester
25	2,4,6-trimethoxytoluene
26	3,4-dimethoxybenzenemethanol, methyl ether
27	Benzenedicarboxylic acid, dimethyl ester (3 isom.)
28	1-(3,4-dimethoxyphenyl)-1-methoxyethane
29	3-chloro-4-methoxybenzenecarboxylic acid, methyl ester
30	1-(3,4-dimethoxyphenyl)-1-propene
31	3,4-dimethoxybenzaldehyde
32	dimethoxybenzenemethanol, methyl ether
33	1-(3,4-dimethoxyphenyl)-2-methoxypropane
34	dimethoxybenzoic acid, methyl ester
35	dimethoxybenzenemethanol, methyl ether
36	3,4-dimethoxyacetophenone
37	3,4,dimethoxybenzoic acid, methyl ester
38	1-(4-methoxyphenyl)-1,2-dimethoxypropane
39	3,4-dimethoxybenzeneacetic acid, methyl ester
40	*trans*- 4-methoxyacrylic acid, methyl ester
41	3,4,5-trimethoxyacetophenone
42	2-methoxy-1,5-benzenedicarboxylic acid, methyl ester
43	3-(3,4-dimethoxyphenyl)propanoic acid, methyl ester
44	3,4,5-trimethoxybenzoic acid, methyl ester
45	1-(3,4-dimethoxyphenyl)-1,2-dimethoxypropane
46	*cis*- 1-(3,4-dimethoxyphenyl)-1,3-dimethoxy-1-propene
47	3,4,5-trimethoxybenzeneacetic acid, methyl ester
48	*trans*- 1-(3,4-dimethoxyphenyl)-1,3-dimethoxy-1-propene

Alternative reagents are used to assist in differentiating the originally free and methylated phenolic groups. Martin *et al.* (*20*) used tetrabutylammonium hydroxide (TBAH) for the structural characterization of lignins, and del Rio *et al.*, (*12*) used TBAH to study the formation of catechols during the coalification process. Pyrolysis in the presence of TBAH introduces a butyl moiety in the originally free hydroxyl group (forming an O-butyl ether) that can thus be distinguished from the original methoxyl groups in the structure of the lignin. Pyrolysis in the presence of TBAH only methylates phenolic groups, while the aliphatic hydroxyls remain unbutylated, probably due to their weakly acidic character. Pyrolysis in the presence of TBAH of the HA isolated from low-rank coals have shown the release of moieties with guaiacyl and syringyl structures having the OH groups at C-3 and C-5 either methylated or butylated. Larger amounts of compounds having the OH groups at C-3 and C-5 butylated were released from the lignite than from the peat, indicating that coalification induces the demethylation of the lignin structure to form catechols which become butylated with TBAH.

Main Advantages of the TMAH/Thermochemolysis Procedure in Comparison with Other Chemical Degradative and Pyrolysis Methods

The application of the TMAH/thermochemolysis procedure has several advantages over classical wet chemical and pyrolytic techniques. First, TMAH thermochemolysis produces more lignin-derived products with intact or partially altered side chains than other procedures such as the CuO oxidation procedure or conventional flash pyrolysis. This allows one to evaluate the degree of preservation of side-chain carbons in the original sample. Since these side-chain carbons are sensitive to environmental modification by biological processes, the TMAH procedure may eventually prove useful for assessing the rate of lignin modification during biological alteration. In comparison to conventional pyrolysis, this method also avoids decarboxylation of preexisting carboxylic moieties and releases aromatic and aliphatic acids as their methyl esters. Methylation also renders many of the polar products volatile enough for chromatographic analysis, which otherwise can be highly biased.

Second, the TMAH procedure methylates lignin phenols which have been microbially modified by demethylation reactions. Demethylation of lignin units by microbial enzymes to produce catechols is well known (*47*). Unfortunately, the CuO oxidation destroys catechols by ring opening, removing them from the analytical window. The TMAH/thermochemolysis, however, methylates catechols, preserving their analytical integrity. Partially demethylated lignin units in HS or other environmental samples will have their catechols preserved and analyzed as a lignin product. This means that the TMAH thermochemolysis may have a capability of detecting lignin-derived products with greater sensitivity and range, especially where microbial degradation is important.

Finally, and probably the most important advantage, the TMAH procedure is easily performed, carrying out the procedure at 250°C-300°C in a glass microreactor constructed of nothing more than a glass ampoule (*31*). This procedure has been applied to several samples such as lignins, fresh and degraded woods, coalified woods and dissolved organic matter (*31-33*), and the release of phenyl propane units having one, two or three methoxyl groups at the propanoid side-chain is an indication that the reactions conditions are sufficient for the hydrolysis of the β-O-4 bonds in the lignin. The presence of intense peaks for the vanillic acid and vanillin derivatives is an indication that this procedure will allow for calculation of acid/aldehyde ratios as is typically done for the CuO oxidation procedure. The ability to perform the TMAH thermochemolysis procedure in glass tubes allows for quantitative measurements by the addition of internal standards.

Conclusions

A new analytical procedure, termed TMAH thermochemolysis, has been used to assess the structural characterization of HS from a variety of samples. Although this procedure has mainly been performed in pyroprobe units at pyrolysis temperatures, it can also be conducted at subpyrolysis temperatures in sealed glass tubes. Therefore, this procedure can be easily implemented in any laboratory having gas chromatographic capabilities, in contrast to other chemolytic or pyrolytic procedures. This is a potentially valuable advantage, because it makes the technique readily available to most geochemical laboratories.

A significant feature of this method is that it may be able to trace lignin inputs where extensive degradation has occurred and resulted in sufficient alteration of lignin to render it undetected by the CuO or pyrolysis procedure. Microbial demethylation of lignin would not impact the TMAH thermochemolysis because the lignin ancestry is preserved in contrast to the CuO procedure which destroys demethylated lignin units. In essence, the TMAH procedure may be a more sensitive indicator of lignin-derived inputs than the more laborious CuO procedure or conventional pyrolysis.

In general, the data demonstrated that this technique provides relatively good preservation of the original carboxyl and hydroxyl groups in lignin phenols units present in the macromolecular structure of HS owing to protection of the functional groups from thermal reactions.

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